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14. ABSTRACT
Ductal carcinoma in situ (DCIS) makes up 18% of all new breast cancer diagnoses, and is considered a precursor to invasive breast cancer even though the majority of cases—almost 70%—may never progress to invasive disease. Markers that identify which patients are most likely to experience progression are critically needed so that fewer patients are over-treated. This study is evaluating two novel tumor markers that may indicate greater risk of tumor progression based on recent work that suggests that stromal syndecan-1 expression induces an extracellular matrix with aligned collagen fiber architecture, and that this collagen alignment in turn facilitates malignant cell invasion. We are using archived tumor tissue from 267 cases of DCIS of the breast to evaluate syndecan-1 expression and collagen alignment. These DCIS cases, diagnosed between 1995 and 1999, have been followed for breast cancer outcomes; 13% of cases have experienced a second breast cancer diagnosis. Analysis suggests that treatment and method of detection are important covariates to include in statistical modeling. Preliminary analysis of collagen alignment and syndecan-1 expression suggests that features of the tumor microenvironment are related with disease-free survival after a diagnosis of DCIS.

15. SUBJECT TERMS
Ductal carcinoma in situ, breast cancer, tumor microenvironment, collagen alignment, syndecan-1, disease-free survival, second harmonic generation

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INTRODUCTION

Ductal carcinoma in situ (DCIS) makes up 18% of all new breast cancer diagnoses, and is considered a precursor to invasive breast cancer. It is estimated that almost 70% of DCIS cases may never progress to invasive disease. However, since the transition from DCIS to invasive breast cancer is a critical progression step associated with a substantial drop in survival, patients are uniformly treated with aggressive therapy, and thus many are being over-treated. Unfortunately, relatively little is known about the factors that govern this progression, and so markers that isolate patients likely to progress have not been identified. An emerging approach in tumor biology focuses on important changes in the stromal tissue surrounding malignant cells during tumor progression. The recent work of Drs. Patricia Keely and Andreas Friedl with invasive breast carcinoma suggests that stromal syndecan-1 expression induces an extracellular matrix with an aligned collagen fiber architecture, and that this collagen alignment in turn facilitates malignant cell invasion. These changes have not been investigated in DCIS. We hypothesize that re-alignment of the extracellular matrix, triggered by syndecan-1 induction in stromal fibroblasts, plays a major role in the progression from DCIS to invasive breast cancer, and thus can be used as a marker to predict outcome. Our objective is to evaluate this hypothesis using archived tumor samples and follow-up data from Dr. Amy Trentham-Dietz's cohort study of 267 DCIS cases with available tumor samples who were recruited upon their diagnosis between 1995 and 1999. Collagen patterns and stromal expression of syndecan-1 will be evaluated from archived unstained tumor slides using state-of-the-art methods by Drs. Keely and Friedl, respectively.

BODY

The approved Statement of Work for this grant includes:

Task 1. **Obtain and maintain regulatory approval, Months 1-24:**

a. Obtain initial IRB/Human Subjects approvals, Months 1-6.

Progress report: Initial IRB/human subjects approval was obtained in August 2011 (month 6) from both the University of Wisconsin (UW) Health Sciences IRB and the DOD Human Research Protection Office (HRPO).

b. Obtain continuing review annual approval from IRB, Month 12.

Progress report: An annual progress report was submitted to the UW Health Sciences IRB. Approval was obtained with the expiration date of 3 May 2013. Another annual progress report was approved by the Health Sciences IRB on 15 April 2013 to expire on 14 April 2014.

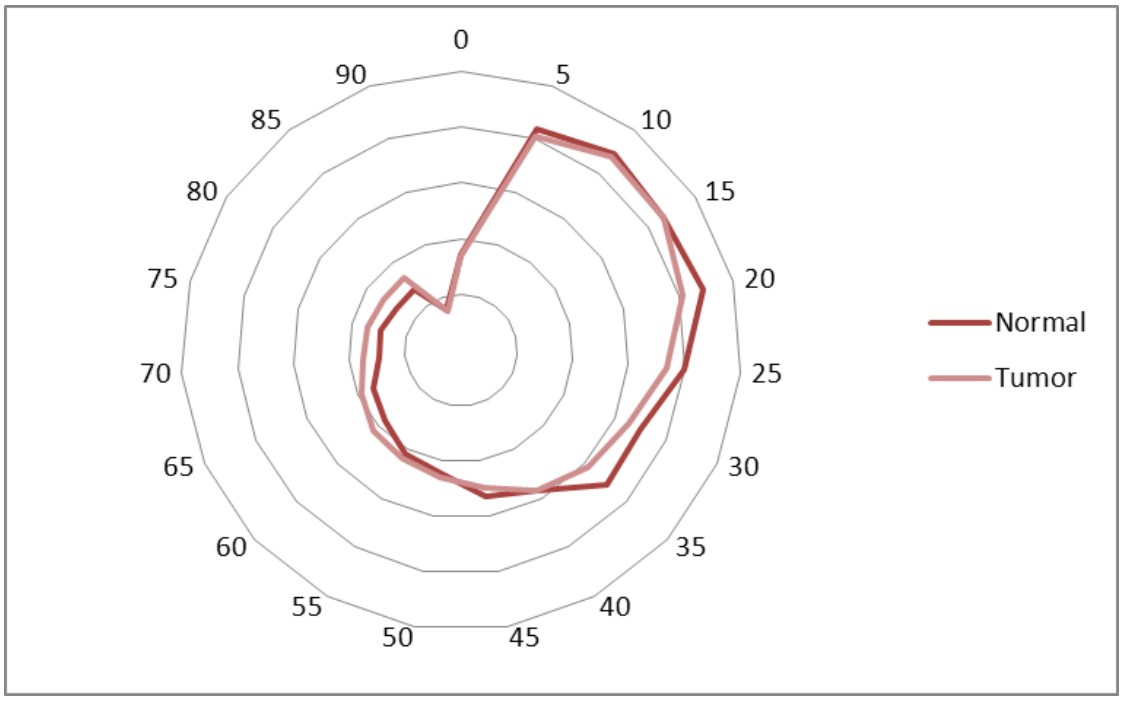
Task 2**. Evaluate tumor microenvironment in 267 DCIS samples, Months 7-10;**

a. Evaluate collagen alignment patterns, Months 5-10.

Progress report: Evaluation of the collagen alignment patterns for the tumor tissue slides has been completed. Collagen fibers were imaged using second harmonic generation (SHG) microscopy, which is a non-linear optical imaging form of microscopy. The technique takes advantage of the unique non-centrosymmetric structure of collagen in combination with the multiphoton absorbance of laser light by the peptide bonds of collagen to act as a frequency doubler. The net effect is that the emitted light is of exactly one-half the

wavelength of the incident light upon interaction with collagen. In this way, an image of the collagen extracellular matrix (specifically) is acquired. These images were then transformed in the frequency space into curvelets, which are essentially vector representations of individual collagen fibers. A boundary between the DCIS lesions and stroma was drawn in the image by the user and software program (CurveAlign2) then measured the angle at which each curvelet crossed the border. These individual measurements were compiled to create a histogram of the angles at which collagen fibers are oriented with respect to the DCIS boundary. Since there are many fibers at any given lesion, this automated analysis is highly useful. The multiphoton microscope and curvelet analysis program used were both custom created through established collaborations here at the University of Wisconsin.

Collagen alignment was evaluated in 3-7 DCIS lesions each for 229 cases. (Collagen alignment could not be evaluated for 38 cases.) We compared the angles at which the collagen fibers were configured relative to the DCIS boundary. A similar assessment was completed for normal tissue from 95 cases. Statistical analysis designed for compositional data demonstrates that the distribution of collagen fiber angles for the DCIS lesions was significantly different than the pattern of collagen fiber angles for the adjacent normal ducts or acini (P=0.01). As shown in the figure below, in both normal and DCIS, many of the fibers were aligned at 5 to 15-degree angles; relatively few of the lesions were aligned at 60 to 85-degree angles, and even fewer fibers were aligned at 90-degree angles. Compared to normal, DCIS lesions had relative increases at 55-85-degree angles. An important point to note is that this tumor cohort included the patients who are not likely to progress with those who will progress. We are currently analyzing the outcome data to determine whether the subset of tumors that demonstrates angles greater than 55 degrees corresponds to disease progression. The other angles show relative drops (fiber alignments were lower for tumor relative to normal for 15 to 40-degree angles).



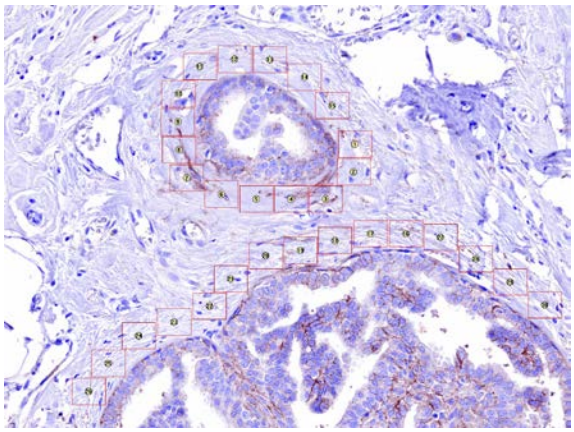
The CurveAlign2 program calculates the mean curvelet angle (collagen alignment measurement #1) in addition to creating the distribution histogram (collagen alignment measurement #2) for each image. The program has recently been updated where it now creates a new image for each round of analysis where individual curvelets are superimposed upon the SHG image and colored based on the angle at which they cross the tumor/stroma boundary. Additionally, spatial information is taken into account when creating this image such that only groups of curvelets in close proximity to each other who share a similar angle are colored; in this way we have created a "heat map" image that easily identifies aligned collagen. We can use information from this new image to further quantify the amount of aligned collagen present in each image (collagen alignment measurement #3).

We have also evaluated by eye whether lesions were characterized by the Tumor Associated Collagen Signature-3 (TACS-3) phenotype (collagen alignment measurement #4), with the presence of radially aligned collagen fibers that are hypothesized to facilitate invasion.(Ref: Provenzano et al. BMC Med 2006: 4:38). Other tumor signatures include the presence of dense collagen around the tumor (TACS-1), and the presence of straightened collagen fibers stretched around the tumor, indicating an increased mechanical load (TACS-2).

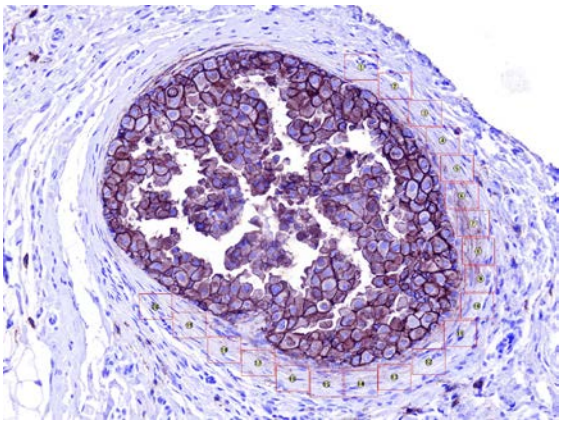
Among the DCIS cases, 49% had tumors that reflected the TACS-3 phenotype.

b. Evaluate syndecan-1 expression, Months 5-10.

Progress report: Following initial optimization, immunohistochemical labeling for syndecan-1 has been completed for all 267 cases. As with collagen alignment, syndecan-1 staining is evaluated in 3-7 lesions for each patient. Quantitative evaluation of the stained tissue slides for syndecan-1 expression is performed by focusing on fibroblasts located in the periductal stroma using the Nuance image analysis system. Regions of interest were placed around the ducts (red boxes shown in the images below), and staining intensity was evaluated quantitatively for each of these regions. Total, average, and maximum intensity are calculated and summarized across each tumor and patient by the Nuance software.



Low syndecan-1 staining intensity



High syndecan-1 staining intensity

While labeling of all slides is complete, quantitative analysis is not yet finished. This task is expected to be completed by early December 2013.

Task 3. **Determine outcome status among 267 DCIS cohort subjects, Months 7-8.**

a. Clean study cohort dataset to determine 2nd breast cancer events, Months 7-8.

Progress report: We have ascertained 32 second breast cancer diagnoses (13%) among 255 DCIS cases.

b. Categorize 2nd breast cancer events by invasive/in situ stage, ipsilateral/contralateral location, and estrogen receptor status, Months 7-8.

Progress report: Among the 32 second diagnoses among the 255 DCIS cases, 34% were invasive breast cancer, 53% were *in situ* breast cancer, and 13% (N=4) have unknown extent of disease (See table). A slight majority of second diagnoses were ipsilateral (53%). Estrogen receptor (ER) status is unknown for most of the second diagnoses (69%); 90% of the second diagnoses with known ER status were ER-positive.

Summary of DCIS cases (median length of follow-up = 11.8 years)

	N	%
Total	255	
Second diagnoses	32	12.5%
Extent of disease		
Invasive	11	34%
In situ	17	53%
Unknown	4	13%
Laterality		
Ipsilateral	17	53%
Contralateral	13	40%
Both	2	6%
Unknown	1	3%
Estrogen receptor status		
Positive	9	28%
Negative	1	3%
Unknown	22	69%

Task 4. **Statistical analyses, Months 11-17:**

a. Link the tumor microenvironment data from Task 2 to the cohort data of Task 3, Month 11.

b. Characterize the collagen alignment patterns and syndecan-1 expression levels in the 267 DCIS samples, Months 11-12.

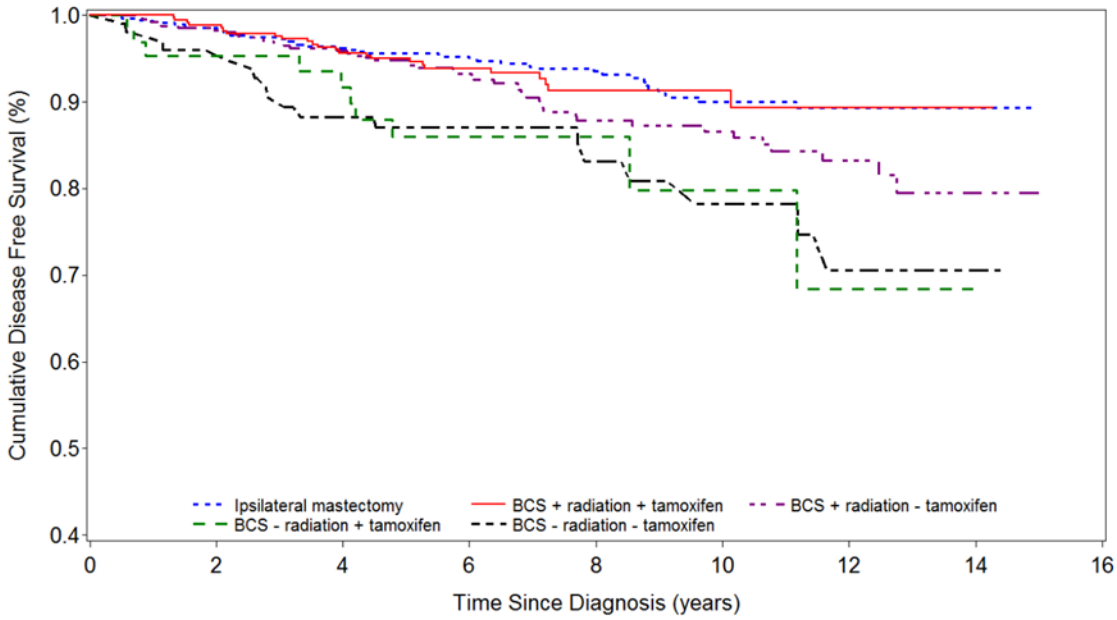
c. Evaluate the association between the tumor microenvironment data and tumor/patient characteristics, Months 13-15.

d. Determine the relation between the tumor microenvironment data and disease-free survival, Months 16-17.

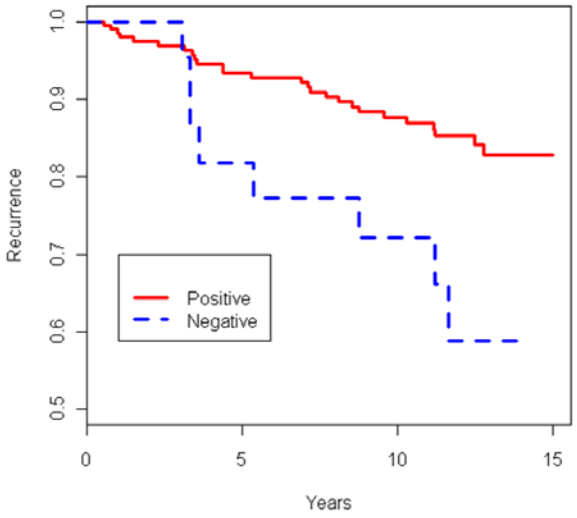
Progress report: Statistical analyses utilizing data for collagen alignment (fiber angles and TACS) of tumor samples has begun; analysis of syndecan-1 expression levels is currently underway. Data collection was completed for collagen alignment measures in March 2013; assessment of syndecan-1 expression will require until December 2013. Delays were due

to challenges with adequate staffing levels. New personnel have joined the team, and all tasks are expected to be completed this year.

We have continued to prepare for statistical analyses by characterizing disease-free survival in the WISC Cohort from which the DCIS cases with tumor tissue samples were drawn. There are 1,959 DCIS cases in the WISC Cohort. After an average of 7.1 years of follow-up, 143 second breast cancer events occurred. We have described disease-free survival in this cohort according to treatment patterns in two presentations and a published manuscript.[1-3] Overall five-year disease-free survival was similar among women treated with ipsilateral mastectomy (95.6%; 95% CI: 93.5, 97.0) compared to women treated with BCS and radiation (94.8%; 95% CI: 92.8, 96.1), though women receiving BCS without radiation experienced poorer overall disease-free survival (87.0%; 95% CI: 80.6, 91.5; see figure below). Among women treated with BCS and radiation, the addition of tamoxifen was associated with a 30% (HR = 0.70; 95% CI: 0.41, 1.19) reduction in risk of second events. Women treated with BCS, radiation, and tamoxifen had comparable risk of a second event as those treated with ipsilateral mastectomy (HR=1.20; 95% CI: 0.71, 2.02).



We have also examined tumor and patient characteristics in relation to DCIS disease-free survival in the WISC Cohort. DCIS cases detected symptomatically were more likely to have a recurrence than cases detected by screening mammography (HR=1.6; 95% CI 0.9-3.0). Tumor size, grade, and histologic subtype were not strongly associated with disease-free survival. Power was inadequate to detect significant differences according to tumor markers including ER and PR although the relations were in the expected direction, with recurrence less likely for tumors positive for expression of these hormone receptors (ER: hazard ratio 0.69, 95% CI 0.27-1.77; PR: hazard ratio 0.53, 95% CI 0.25-1.15).[4] Similarly, Ki-67 (hazard ratio 1.54, 95% CI 0.73-3.25) and HER-2/neu (hazard ratio 1.05, 95% CI 0.43-2.56) expression were not related to recurrence. However, analysis suggested that cases with tumors positive for p53 expression were less likely to experience recurrence (hazard ratio 0.48, 95% CI 0.19-1.21) with cases positive for p53 expression tending to experience a longer time to recurrence (log-rank p-value = 0.01; see survival curve below).



Time to recurrence according to p53 expression among DCIS cases

In our study, there was a suggestion that disease-free survival appeared to vary little according to pre- and post-diagnosis patient factors including age, family history of breast cancer, body mass index, physical activity, parity, postmenopausal hormone use, and education (see table below).[5-6] However, women who consumed greater amounts of alcoholic beverages after diagnosis appeared to have a slightly greater risk of recurrence (P-trend=0.02).

Hazard ratios and 95% confidence intervals for the association between lifestyle factors and the risk of recurrence, DCIS cohort, 1997-2010

		Pre-diagnosis		Post-diagnosis	
	Recurrences	HR (95% CI)	P_{trend}	HR (95% CI)	P_{trend}
BMI (kg/m2)					
<18.5	2	1.27 (0.31, 5.12)	0.14	0.98 (0.28, 3.45)	0.13
18.5-24.9	76	Ref		Ref	
25-29.9	57	1.10 (0.76, 1.57)		1.03 (0.65, 1.64)	
30+	27	0.82 (0.52, 1.30)		1.23 (0.56, 2.68)	
Continuous; Mean (std)	162	0.99 (0.96, 1.02)		1.03 (0.97, 1.10)	
Total Physical Activity (hrs/week)					
No activity	44	Ref	0.66	Ref	0.90
>0 - 2	37	0.97 (0.62, 1.52)		0.37 (0.12, 1.07)	
>2 - 5	54	1.16 (0.77, 1.75)		0.92 (0.47, 1.81)	
5+	27	0.81 (0.49, 1.33)		0.89 (0.36, 2.22)	
Continuous; Mean (std)	162	0.98 (0.94, 1.02)		0.97 (0.88, 1.08)	
Total Alcohol (drinks/week)					
No alcohol	27	Ref	0.88	Ref	0.02
>0 - <2	80	0.98 (0.62, 1.53)		1.28 (0.59, 2.78)	
2 - <7	35	0.91 (0.54, 1.53)		1.76 (0.64, 4.80)	
7+	20	1.04 (0.58, 1.88)		2.59 (0.61, 11.0)	
Continuous; Mean (std)	162	1.02 (0.99, 1.05)		1.03 (0.94, 1.11)	

Pre-diagnosis N=1925; Post-diagnosis N=1903 (BMI), N=1011 (Physical Acticity, Alcohol)

Adjusted for age at diagnosis, menopausal status, mode of detection, treatment type, postmenopausal hormone use, tamoxifen use, year of diagnosis, tumor size, tumor grade, and remaining lifetyle factors

Post-diagnosis results are additionally adjusted for pre-diagnosis levels of each lifestyle factor

P value is for linear trend across categories of each lifestyle factor

These results provide information as to the inclusion of potentially confounding factors in the analyses of Task 4.d. Our findings suggest that treatment received and mode of detection are important variables for inclusion in multivariable models of tumor microenvironment in relation to disease-free survival. The overall disease-free survival rates in the parent cohort will also be informative in comparing the disease-free survival rates observed among the DCIS cases with tumor tissue samples (i.e., to guide the generalizability and interpretation of the results).

Preliminary analysis has evaluated recurrence in the DCIS according to the presence of TACS-3 in the tumor tissue samples. Of cases with zero lesions with TACS-3, 88.7% did not have a recurrence and 11.3% did have a recurrence. Of cases with any lesions with TACS-3, 83.9% did not have a recurrence and 16.1% did have a recurrence (P-value=0.3). Although not statistically significant, this preliminary analysis suggests that certain collagen alignment patterns may be associated with increased likelihood for disease progression.

***Task 5*. Communication of results, Months 12-24:**

a. Submit annual progress report to the DOD, Month 12
b. Prepare manuscripts describing the results found in Task 4, Months 18-24.
c. Present the study results at the DOD Era of Hope meeting and other national conferences, Months 12-24.
d. Deliver final report to the DOD, Month 24.

Progress report: The first annual progress report was approved 13 June 2012. A six-month no-cost extension was approved 8 February 2013, and the second progress report was approved 26 April 2013. This report serves as the final report.

Communication of results has begun including presentations at three national scientific conferences[1,4,5] and one regional conference.[2] As described under Task 4, two manuscripts have been submitted – one published,[3] one under review[6] – as preparation for the analysis of tumor microenvironment in relation to disease-free survival. A statistical analysis plan has been prepared so that analysis can be conducted quickly after all data are available. Preliminary results for collagen alignment will be presented at the CTRC-AACR San Antonio Breast Cancer Symposium during 10-14 December 2013 (Poster session 1, Program Number P1-06-06; abstract attached). An abstract is in preparation to submit by 20 November 2013 for presentation at the annual meeting of the American Society of Preventive Oncology describing the results for synedan-1 and collagen alignment in relation to patient factors and disease-free survival; the meeting is planned for 8-11 March 2014.

A comprehensive manuscript describing the primary results from this study will be prepared for publication concurrently with presentation at national scientific meetings in spring 2014.

KEY RESEARCH ACCOMPLISHMENTS

- IRB approval obtained and maintained
- Procedures finalized for evaluating collagen fiber alignment in DCIS samples including imaging and quantification of angles as well as qualitative assessment (TACS)
- Collagen alignment for all tumor and normal tissue samples was completed 4 March 2013

- Tumor slides have been stained for syndecan-1 expression
- Quantitative assessment of syndecan-1 expression has begun and is expected to be completed by 31 December 2013
- Second breast cancer diagnoses (invasive and *in situ*) have been identified among cohort participants, and the relations between disease-free survival, treatment patterns, and patient factors have been described in publications and at scientific conferences
- Methods for statistical analysis have been established and preliminary data analysis has begun
- An abstract has been accepted for presentation at the annual CTRC-AACR San Antonio Breast Cancer Symposium in December 2013

REPORTABLE OUTCOMES

Preliminary findings regarding DCIS disease-free survival in the parent WISC Cohort were presented as a poster at the annual meetings of the AACR's Frontiers in Cancer Prevention Research.[1] Findings have also been disseminated as oral presentations at the annual breast cancer conference at the Vermont Cancer Center[2] and the annual meetings of the Society for Epidemiologic Research[4] and the American Society of Preventive Oncology.[5] One manuscript is now published;[3] another paper has been submitted for publication and is under review.[6] Another poster will be presented this December at the CTRC-AACR San Antonio Breast Cancer Symposium (see appendix).

Based on procedures established in this project, we successfully obtained a new grant from the NIH ("Vermont PROSPR Research Center", U54 CA163303); collaborators are now members of the Population-based Research Optimizing Screening through Personalized Regimens (PROSPR) consortium organized by the Applied Research Program within the Division of Cancer Control and Population Sciences at the NIH.

CONCLUSION

All study procedures have been finalized. Human subjects' protection approval for this study was obtained August 2011 from the University of Wisconsin Institutional Review Board, and on-going approval has been maintained. Using H&E stained slides, tumors were imaged using second harmonic generation microscopy and fiber alignment patterns were evaluated for 229 cases. Preliminary analysis suggests that fiber alignment patterns are significantly different for DCIS lesions than for adjacent normal duct cells. Tumor slides have been stained for syndecan-1, and assessment of staining intensity has begun. Statistical data analysis of the study aims will ensue upon completion of the assessment of collagen fiber alignment patterns in both tumor and normal tissue using several different measures of collagen alignment and syndecan-1 expression. Since data collection is not complete, no scientific knowledge or reportable outcomes have been produced yet, although preliminary data analysis has begun and methods sections of manuscripts are being drafted. While we have experienced some delays with establishing procedures for evaluating fiber alignment and evaluating syndecan-1 expression, and one microscope did stop functioning, methods are firmly in place and we expect to achieve the proposed Tasks within the project period since a 6-month no-cost extension has been approved.

REFERENCES

[1]Sprague B, McLaughlin V, Hampton J, Newcomb P, Trentham-Dietz A. DCIS disease-free survival in the population-based Wisconsin In Situ Cohort. [Abstract] Cancer Prevention Research 4(10 Suppl): A28, 2011. Poster presentation at the Tenth AACR International Conference on Frontiers in Cancer Prevention Research, October 22-25, 2011, Boston, MA.

[2]Sprague BL. Predictors of recurrence after a DCIS diagnosis. Oral presentation at the Vermont Cancer Center 15th Annual Breast Cancer Conference, October 5, 2012, Burlington, VT.

[3]Sprague BL, McLaughlin V, Hampton J, Newcomb P, Trentham-Dietz A. Disease-free survival by treatment after a DCIS diagnosis in a population-based cohort study. Breast Cancer Res Treat 141(1): 145-54, 2013.

[4]Binder A, Hampton J, Sprague B, Walsh M, Friedl A, Newcomb P, Trentham-Dietz A. tumor markers in relation to disease-free survival among women with ductal carcinoma in situ of the breast. [Abstract] Am J Epidemiol 177(11 Suppl): S121, 2013. Oral presentation at the 46th Annual Meeting of the Society for Epidemiologic Research, June 18-21, 2013, Boston, MA.

[5]McLaughlin V, Trentham-Dietz A, Hampton JM, Newcomb PA, Sprague BL. Lifestyle factors and the risk of a second breast cancer diagnosis after DCIS in the Wisconsin In Situ Cohort. [Abstract] Cancer Epidemiol Biomarkers Prev 22(3): 472, 2013. Oral presentation at the 37th Annual Meeting of the American Society of Preventive Oncology, March 10-12, 2013, Memphis, TN.

[6]McLaughlin VH, Trentham-Dietz A, Hampton JM, Newcomb PA, Sprague BL. Lifestyle factors and the risk of a second breast cancer after ductal carcinoma in situ. Cancer Epidemiol Biomarkers Prev (under review as of 9/4/2013).

APPENDICES

1. Poster[1]
2. Reprint: Sprague BL, McLaughlin V, Hampton J, Newcomb P, Trentham-Dietz A. Disease-free survival by treatment after a DCIS diagnosis in a population-based cohort study. Breast Cancer Res Treat 2013 Aug; 141(1): 145-54.[3]
3. Published abstract[4]
4. Published abstract[5]
5. Accepted abstract for presentation at the annual CTRC-AACR San Antonio Breast Cancer Symposium in December 2013

DCIS Disease-Free Survival in the Population-Based Wisconsin In Situ Cohort

Vicki McLaughlin[1,2], Brian Sprague[1], John Hampton[3], Polly Newcomb[4], Amy Trentham-Dietz[3]

[1]University of Vermont, [2]University of Massachusetts Amherst, [3]University of Wisconsin, [4]Fred Hutchinson Cancer Research Center

Introduction

• Ductal carcinoma in situ (DCIS) is the earliest form of breast cancer. DCIS constitutes 20% of all new breast cancer diagnoses and every year over 50,000 women are diagnosed with DCIS in the United States.

• A number of randomized trials have demonstrated the effectiveness of radiation and tamoxifen in reducing the risk of second events after a DCIS diagnosis. However, few population-based studies have examined disease-free survival according to treatment in community practice.

Study Aim

We describe the risk of second breast cancer events among women diagnosed with DCIS in the state of Wisconsin over the past 15 years. We determine overall disease-free survival among treatment groups and examine the frequency of ipsilateral and contralateral second events.

Methods

The Wisconsin In Situ Cohort (WISC) study was designed to evaluate breast cancer outcomes among a large population-based cohort of women with in situ breast cancer.

Eligible
Women age 20-74 years with a first primary diagnosis of breast carcinoma in situ reported to the Wisconsin Reporting System 1995-2006

↓

Enrolled
78% of eligible women (N=2,281)

↓

Study Population
Women diagnosed with DCIS (N=1,959)

↓

Final Analytic Sample
Women with DCIS and complete treatment information (N=1,689)

Treatment Information

Surgical, radiation, and hormone treatment was self-reported at a baseline interview approximately 1 year after diagnosis and during follow-up interviews conducted every 2 years.

Breast Cancer Recurrence

Recurrences were self-reported at follow-up interviews conducted at two year intervals and were confirmed via pathology reports.

Statistical Methods

Descriptive statistics were used to compare baseline characteristics among women with each treatment type. Unadjusted disease-free survival was determined using Kaplan-Meier survival estimates. Cox proportional hazards regression was used to estimate hazard ratios by treatment, adjusting for confounders.

Results

Baseline Characteristics

The median age at the time of diagnosis was 56.0 years and most women in the study were postmenopausal (59.2%). A majority of breast cancers were detected via mammography (85.4%). Use of tamoxifen was reported by 38.0% of women, most frequently among women undergoing breast conserving surgery (BCS) (Figure 1).

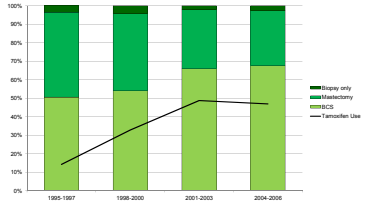


Figure 1: Surgical treatment rates and tamoxifen use by year of diagnosis, Wisconsin DCIS Cohort, 1995-2010

Risk of Recurrence

Over a median follow-up of 6.3 years, 133 second breast cancer events were recorded. The distribution of these recurrences by laterality is shown in Figure 2. Estimated hazard ratios for the risk of a second event by treatment group are shown in Table 1. The risk of a second ipsilateral event was significantly higher for women treated with BCS without radiation or biopsy only.

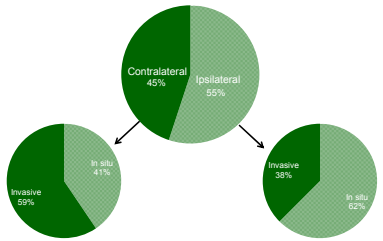


Figure 2: Distribution of second breast cancer events, Wisconsin DCIS Cohort, 1995-2010

Table 1: Estimated hazard ratios for the risk of 2nd breast cancer events according to treatment group, Wisconsin DCIS Cohort, 1995-2010.

		Any second event			Any ipsilateral event			Invasive ipsilateral events			In situ ipsilateral events		
	No. Total	No. events	HR [a]	95% CI	No. events	HR [a]	95% CI	No. events	HR [a]	95% CI	No. events	HR [a]	95% CI
Treatment type													
BCS with radiation [b]	861	63	1	-	27	1	-	7	1	-	10	1	-
BCS without radiation [b]	181	20	1.44	0.86, 2.40	14	2.24	1.15, 4.35	3	1.84	0.45, 7.54	5	2.25	0.74, 6.86
Ipsilateral mastectomy	526	43	0.83	0.54, 1.26	13	0.52	0.26, 1.05	0	-	-	2	0.25	0.05, 1.19
Bilateral mastectomy	75	0	-	-	0	-	-	0	-	-	0	-	-
Biopsy only	46	7	1.82	0.82, 4.06	6	3.13	1.24, 7.86	2	4.42	0.81, 24.2	3	4.01	0.99, 16.2

[a] Multivariable model adjusts for age group, menopausal status, screening history, mode of detection, tumor size, grade, tamoxifen use, and year of diagnosis. Unknown values of covariates were estimated using multiple imputation (m=10).
[b] BCS = Breast conserving surgery.

Results

Disease-free Survival

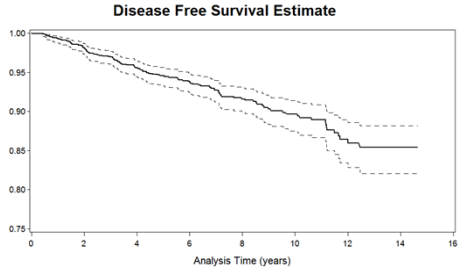
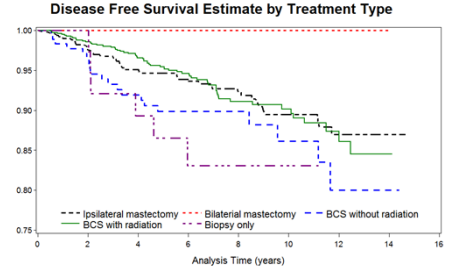


Figure 3: Overall disease-free survival with 95% confidence bands, Wisconsin DCIS Cohort, 1995-2010.



Five-year DFS (95% CI) by Treatment		
Bilateral mast.	100	-
Ipsilateral mast.	94.7	(92.3, 96.3)
BCS plus radiation	95.4	(93.7, 96.7)
BCS	89.9	(84.0, 93.7)
Biopsy only	86.5	(70.6, 94.2)

Figure 4: Disease-free survival by treatment type, Wisconsin DCIS Cohort, 1995-2010.

Discussion

- In this large population-based cohort study, we observed high disease-free survival rates among women diagnosed with DCIS. At five years after diagnosis, 94.6% of subjects had not experienced a second breast cancer event.
- The highest rates of disease-free survival were observed in women treated with bilateral mastectomy (100%) and the lowest rates in women treated with biopsy only (86.5%). Tamoxifen use reduced the risk of a second event by about 20% (data not shown).
- Limitations of this study included lack of ER/PR status and reliance on self-reported treatment.
- In general we found that the effectiveness of radiation therapy and tamoxifen in reducing risk of second events was comparable to that observed in randomized trials. The results of this study provide population-based data that can be used to guide treatment for DCIS.

Acknowledgements

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EPIDEMIOLOGY

Disease-free survival by treatment after a DCIS diagnosis in a population-based cohort study

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Abstract Randomized trials have demonstrated the efficacy of radiation and tamoxifen in reducing risk of second events after breast-conserving surgery (BCS) for ductal carcinoma in situ (DCIS), but the comparative effectiveness of mastectomy, BCS, and adjuvant treatments have not been established in community practice. We examined disease-free survival (DFS) among 1,676 DCIS cases diagnosed during 1995–2006 in the population-based Wisconsin In Situ Cohort study. Information on patient and tumor characteristics, treatments, and second breast cancer events were collected via a comprehensive review of data from patient interviews, the statewide cancer registry, and pathology reports. Breast cancer DFS was evaluated according to treatment while adjusting for patient and tumor characteristics. After an average of 7.1 years of follow-up, 143 second breast cancer events occurred. Overall 5-year DFS was similar among women treated with ipsilateral mastectomy (95.6 %; 95 % CI 93.5–97.0) compared to women treated with BCS and radiation (94.8 %; 95 % CI 92.8–96.1), though women receiving BCS without radiation experienced poorer overall DFS (87.0 %; 95 % CI 80.6–91.5). Women treated with tamoxifen in addition to BCS and radiation had a similar risk of a second breast event, although the hazard ratio (HR) suggested a potential benefit (0.70, 95% CI 0.41-1.19). Women treated with BCS, radiation, and tamoxifen had comparable risk of a second event as those treated with ipsilateral mastectomy (HR = 1.20; 95 % CI 0.71–2.02). In this population-based sample, the use of BCS with radiation and tamoxifen resulted in high DFS rates comparable to those achieved by ipsilateral mastectomy.

Keywords Ductal carcinoma in situ · Disease-free survival · Breast cancer · Treatment

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Introduction

Ductal carcinoma in situ (DCIS) is the earliest detectable form of breast cancer, in which the malignant cells are confined within the basement membrane of the breast ductal system [1]. A dramatic increase in the incidence of DCIS began in the mid-1980s, mirroring the rise in screening mammography [2]. While the incidence of invasive breast cancer has declined over the past decade, diagnoses of DCIS have continued to rise [3]. DCIS now constitutes more than 20 % of all new breast cancers diagnoses, and every year over 60,000 women are diagnosed with DCIS in the United States [4].

DCIS is considered a true precursor to invasive breast cancer [5], but the natural history of DCIS is poorly understood [6]. Analyses of SEER registry data suggest that less than 2 % of women with DCIS will die from breast cancer within 10 years of their diagnosis [7] and less

than 5 % after about 28 years of follow-up [8]. The high survival rate likely reflects both the availability of effective treatments as well as the relatively indolent nature of most DCIS [9]. Randomized trials have demonstrated that radiation therapy and tamoxifen both improve disease-free survival (DFS) when added to breast-conserving surgery (BCS) [10–13]. While mastectomy and BCS for DCIS have never been compared in a randomized trial, a meta-analysis of clinic-based observational studies of DCIS diagnosed in 1960s through the early 1990s suggested that local recurrence rates were substantially lower among women treated with mastectomy [14].

The variation in DFS according to treatment regimens among more recently diagnosed DCIS cases in community practice is much less clear. Substantial changes in DCIS detection, pathology, surgery, and treatment patterns have occurred over the past 15 years [6]. Data from population-based studies of outcomes after DCIS during this time period are particularly sparse. The Wisconsin In Situ Cohort (WISC) study was designed to evaluate breast cancer outcomes among a large population-based cohort of women newly diagnosed with in situ breast cancer. In this paper, we describe the risk of second breast cancer events among women diagnosed with DCIS between 1995 and 2006. We examined the relative frequency of ipsilateral and contralateral second events, and evaluated DFS among the various treatment groups.

Methods

Study population

The WISC study includes 1,925 women between the ages of 20–74 years with a new first primary diagnosis of DCIS diagnosed during 1995–2006, as reported to the mandatory statewide Wisconsin Cancer Reporting System cancer registry. All such cases were eligible for participation, regardless of the type of facility (academic medical center, community hospital, etc.) in which they were treated. This includes 838 cases who participated in a case–control study [15] during 1997–2001 and 1,087 additional cases recruited during 2002–2006. The aim of the case–control study was to compare risk factors for invasive and in situ breast cancer; the purpose of enrolling both waves of DCIS cases was to examine predictors of DFS. Subject eligibility and recruitment has been previously described in detail [16]. In brief, participation was limited to women with known dates of diagnosis, a listed telephone number, and the ability to complete a telephone interview. Eligibility criteria for the parent case–control study and the subsequent cohort study of DCIS cases were the same. Of all eligible cases, 78 % enrolled in the study. All subjects provided verbal informed consent and the study was approved by the University of Wisconsin Health Sciences Institutional Review Board.

Data collection

Baseline and follow-up interviews were conducted to collect patient data [16]. The baseline telephone interview was conducted approximately 1.3 years after diagnosis (interquartile range 1.0–1.5 years). Follow-up interviews began in 2003 and were conducted at approximately 2-year intervals. These interviews were conducted by telephone until 2010 when a mailed survey was used. Overall, 79.7 % of cases completed at least one follow-up interview or survey.

Data on demographics, reproductive history, and cancer screening history were self-reported by participants during these interviews. Treatment history was collected during the baseline interviews beginning in 2002, and a full treatment history was updated for all subjects during follow-up interviews. Treatment information was not collected at baseline interviews for cases who were recruited during 1997–2001 for the case control study; thus, treatment information is missing for these women if they did not complete a follow-up interview ($N = 161$; Fig. 1). Treatment information was missing for an additional 39 women despite participation in an interview in which this information was asked. The cases missing treatment information were slightly older than other participants (mean age 58.3 versus 55.9 years, $P < 0.001$) but no other significant differences were observed in demographics, reproductive history, or cancer screening history.

The Wisconsin Cancer Reporting System cancer registry provided pathology data on the baseline DCIS diagnosis, including tumor size, grade, and laterality, as well as date of diagnosis. Data on tumor size and grade were missing for 62 and 48 % of cases, respectively (see Table 1).

At each interview, women were asked to report all breast cancer diagnoses since their initial diagnosis. Signed consent was requested from the participants to obtain pathology reports for new diagnoses, and these reports were obtained from the diagnosing hospitals and clinics to confirm these second diagnoses. From these reports, information was collected on laterality and stage (benign, in situ, or invasive diagnosis). A single pathologist reviewed pathology reports to confirm the nature of the diagnosis. Of the 101 self-reported second events for which medical records were available, 92 were confirmed as in situ or invasive (91 %). The nine self-reported second events that were directly contradicted by medical records were not included as second events in this analysis. An additional 42 self-reported second events for which medical records were unavailable were included, for a total of 143 second events. Medical records were unavailable when study subjects refused permission to obtain their pathology

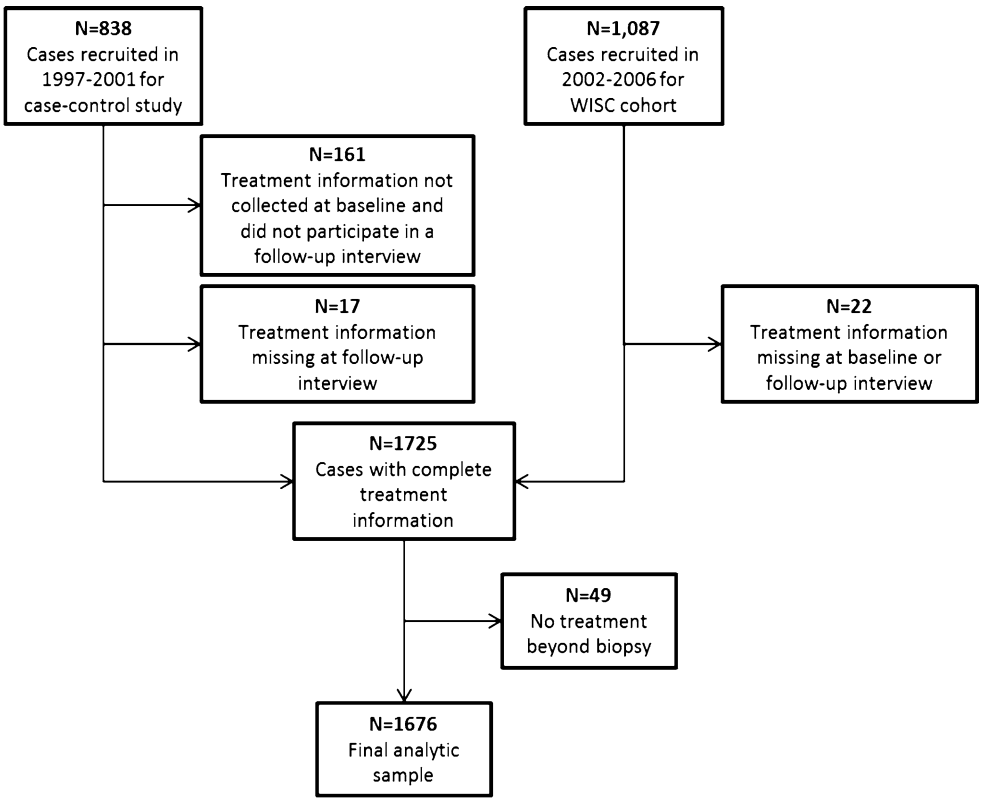

Fig. 1 Flowchart of recruitment and exclusions for the study population, Wisconsin DCIS cases, 1995–2006

reports (consent to this portion of the study was optional) or when the facility was unable or unwilling to disclose the records.

Statistical analysis

We excluded women with missing treatment information ($n = 200$) and women reporting no treatment beyond biopsy ($n = 49$), leaving a final sample size of 1,676 (Fig. 1). Chi-square tests were used to examine whether surgical treatment received varied according to patient and tumor factors. Unadjusted DFS rates for each of these treatment types were estimated using Kaplan–Meier survival estimates with 95 % confidence limits. DFS time in days was defined for each woman as the time from baseline breast cancer diagnosis to date of second event or date of last study contact. For subset analyses, cases with second events missing laterality information (6 %) and/or invasive versus in situ information (29 %) were censored at the time of second event.

All analyses were conducted using SAS statistical software version 9.2. Hazard ratios by surgical treatment, radiation, and tamoxifen status were estimated using Cox proportional hazards regression. Crude models were established with adjustment for age at diagnosis and year of diagnosis only. Multivariable models were additionally adjusted for potentially confounding factors that were selected a priori based on previous literature: menopausal status, screening history, mode of detection, tumor size, grade, and use of raloxifene and aromatase inhibitors. Unknown values of these covariates were estimated with multiple imputation using the Markov Chain Monte Carlo method with ten imputations [17]. The imputation model included all variables listed above from the multivariable model, as well as education level, smoking status, age at first birth, age at menarche, parity, family history of breast cancer, and current use of postmenopausal hormones.

The primary analyses were conducted using the imputed data. Due to the large amount of missing data for tumor size and grade, sensitivity analyses were performed by including only women with complete tumor size and grade information, and by excluding tumor size and grade from the multivariable model. Since BMI may be associated with breast cancer prognosis and may influence treatment decisions, the multivariable analysis was also repeated with adjustment for BMI to assess the impact of this factor.

Results

Selected characteristics of the study sample are summarized by treatment type in Table 1. The median age of the total study population at the time of diagnosis was 55.1 years, and most women in the study were postmenopausal (58.5 %). About 37 % of subjects reported use of tamoxifen, which was less frequent among women

Table 1 Distribution of patient and tumor characteristics by treatment type ($n = 1{,}676$), Wisconsin DCIS cases, 1995–2010

	Overall $N = 1{,}676$ *n* (%)	Bilateral mastectomy $N = 81$ *n* (%)	Ipsilateral mastectomy $N = 593$ *n* (%)	BCS with radiation $N = 826$ *n* (%)	BCS without radiation $N = 176$ *n (%)*	*P* value[a]
Age at diagnosis (years)						<0.001
20–44	205 (12.2)	22 (27.2)	89 (15.0)	77 (9.3)	17 (9.7)	
45–54	626 (37.3)	38 (46.8)	199 (33.5)	319 (38.6)	70 (39.8)	
55–64	517 (30.9)	16 (19.8)	186 (31.4)	275 (33.3)	40 (22.7)	
65–74	328 (19.6)	5 (6.2)	119 (20.1)	155 (18.8)	49 (27.8)	
Menopausal status						0.001
Premenopausal	555 (33.1)	39 (48.1)	214 (36.1)	243 (29.4)	59 (33.5)	
Postmenopausal	981 (58.5)	34 (42.0)	337 (56.8)	506 (61.3)	104 (61.3)	
Unknown	140 (8.4)	8 (9.9)	42 (7.1)	77 (9.3)	13 (7.4)	
Race/ethnicity						0.75
White	1593 (95.0)	76 (93.8)	568 (95.8)	786 (95.2)	163 (92.6)	
Non-white	73 (4.4)	4 (4.9)	23 (3.9)	36 (4.4)	10 (5.7)	
Unknown	10 (0.6)	1 (1.3)	2 (0.3)	4 (0.4)	3 (1.7)	
Pre-diagnosis BMI (kg/m^2)						0.01
<18.5	20 (1.2)	7 (8.6)	4 (0.7)	5 (0.6)	4 (2.3)	
18.5–24.9	763 (45.5)	40 (49.4)	299 (50.4)	355 (43.0)	69 (39.2)	
25.0–29.9	533 (31.8)	20 (24.7)	163 (27.5)	285 (34.5)	54 (36.9)	
≥30.0	346 (20.6)	14 (17.3)	122 (20.6)	176 (21.3)	34 (19.3)	
Unknown	14 (0.8)	0	5 (0.8)	5 (0.6)	4 (2.3)	
Mode of detection						<0.001
Screening mammography	1,438 (85.8)	53 (65.4)	490 (82.6)	747 (90.4)	148 (84.1)	
Other	229 (13.7)	28 (34.6)	100 (16.9)	77 (9.3)	24 (13.6)	
Unknown	9 (0.5)	–	3 (0.5)	2 (0.2)	4 (2.3)	
Tumor size						<0.001
≤1.0 cm	359 (21.4)	11 (13.6)	70 (11.8)	207 (25.1)	71 (40.3)	
1.1–2.0 cm	164 (9.8)	5 (6.2)	53 (9.0)	91 (11.0)	15 (8.5)	
2.1 + cm	118 (7.0)	5 (6.2)	66 (11.1)	41 (5.0)	6 (3.4)	
Unknown	1,035 (61.8)	60 (74.1)	404 (68.1)	487 (59.0)	84 (47.7)	
Grade						<0.001
Low	197 (11.8)	8 (9.9)	53 (8.9)	95 (11.5)	41 (23.3)	
Intermediate	331 (19.8)	14 (17.3)	91 (15.3)	183 (22.2)	43 (24.4)	
High	346 (20.6)	22 (27.2)	157 (26.5)	152 (18.4)	15 (8.5)	
Unknown	802 (47.8)	37 (45.7)	292 (49.2)	396 (47.9)	77 (43.8)	
Tamoxifen use[b]						<0.001
No	984 (58.7)	65 (80.2)	392 (66.1)	424 (51.3)	103 (58.5)	
Yes	616 (36.8)	12 (14.8)	181 (30.5)	358 (43.3)	65 (36.9)	
Unknown	76 (4.5)	4 (4.9)	20 (3.4)	44 (5.3)	8 (4.5)	
Raloxifene use[b]						
No	1573 (93.8)	74 (91.4)	554 (93.4)	781 (94.6)	164 (93.2)	0.405
Yes	53 (3.2)	1 (1.2)	23 (3.9)	22 (2.7)	7 (4.0)	
Unknown	39 (2.3)	6 (7.4)	16 (2.7)	23 (2.8)	5 (2.8)	
Aromatase inhibitor use[b]						
No	1569 (93.6)	78 (96.3)	561 (94.6)	760 (92.0)	170 (96.6)	0.075
Yes	68 (4.1)	–	21 (3.5)	42 (5.1)	5 (2.8)	
Unknown	39 (2.3)	3 (3.7)	11 (1.9)	24 (2.9)	1 (0.6)	

BCS breast-conserving surgery

[a] *P* value provides an unadjusted comparison of treatment groups for records with known covariate status using χ^2 tests

[b] Excludes medication use before the initial diagnosis and after a second breast cancer event

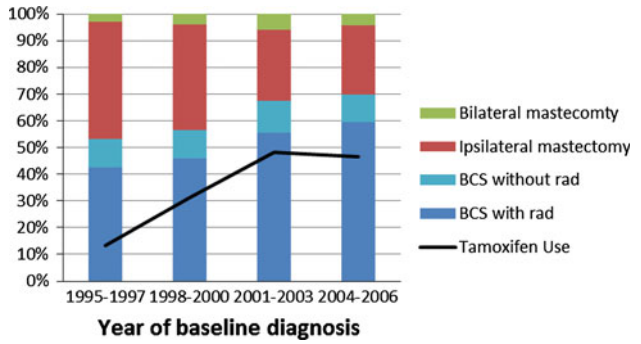

Fig. 2 Surgical treatment, radiation, and tamoxifen use by year of diagnosis, Wisconsin DCIS, cases ($n = 1{,}676$), 1995–2006

undergoing mastectomy compared to those treated with BCS ($P < 0.001$). Approximately 3 % of the study population reported use of raloxifene and 4 % reported use of aromatase inhibitors.

Use of BCS increased over time during the study period, while use of mastectomy declined (Fig. 2). Mastectomy was more frequent among younger, premenopausal women, women whose tumors were detected by a method other than routine mammography, and women with larger or higher grade tumors (Table 1). Among women treated with BCS, the use of radiation increased modestly over time (Fig. 2). Tamoxifen use increased substantially during the study period. Of women diagnosed in 1995–1997, 13.2 % used tamoxifen, compared to 46.5 % of women diagnosed in 2004–2006 (Fig. 2).

Over an average of 7.1 years (range 0.4–15.1 years) of follow-up time, 143 second breast cancer events occurred. Characteristics of the second events according to surgical treatment group and radiation are described in Table 2. The second events were fairly evenly split between ipsilateral and contralateral events (52 vs. 48 % of those with known laterality), and in situ and invasive events (52 vs. 48 % of those with known stage). The majority of second events among women with an ipsilateral mastectomy were in the contralateral breast, whereas ipsilateral events were more common among women treated with BCS without radiation. Second events were evenly split between ipsilateral and contralateral among women treated with BCS and radiation. There were no second events experienced among women who were treated with bilateral mastectomy.

Figure 3a displays the Kaplan–Meier curve for DFS among the DCIS cohort. DFS according to treatment type is shown in Fig. 3b and 5-year DFS rates are displayed in Table 3. The overall 5-year DFS rate for the cohort was 94.5 % (95 % CI 93.2–95.5 %), and ranged from 87 % for women treated with BCS without radiation to 100 % for women undergoing a bilateral mastectomy. Overall 5-year DFS was similar among women undergoing ipsilateral mastectomy (95.6 %) and BCS with radiation (94.8 %). Differences in overall DFS by treatment were driven mainly by variation in rates of ipsilateral events. Five-year DFS for contralateral events was above 96 % regardless of treatment type.

Multivariable hazard ratios for second events are displayed in Table 4. Compared to women treated with ipsilateral mastectomy, the risk of a second event for women treated with BCS without radiation was more than twice as high (HR 2.66, 95 % CI 1.58–4.46). The addition of radiation to BCS was associated with a 41 % reduction in risk of second events (HR 0.59, 95 % CI 0.38–0.92; data not shown). Compared to women treated with ipsilateral mastectomy, the risk of a second event was higher among the group of women treated with BCS and radiation (HR = 1.61; 95 % CI 1.07–2.43; Table 4). Within this group, risk of a second breast cancer event was similar according to tamoxifen use, although the hazard ratio suggested a potential benefit (HR 0.70; 95 % CI 0.41–1.19;

Table 2 Distribution of second breast cancer events among 1,676 Wisconsin DCIS cases, 1995–2010

	Overall	Ipsilateral mastectomy	BCS with radiation	BCS without radiation
Any second event, *N* (%)	143 (100)	41 (100)	74 (100)	28 (100)
Ipsilateral events	70 (48.9)	12 (29.3)	35 (47.3)	23 (82.1)
In situ	30 (21.0)	0	17 (23.0)	13 (46.4)
Invasive	18 (12.6)	3 (7.3)	6 (8.1)	9 (32.1)
Unknown	22 (15.4)	9 (21.9)	12 (16.2)	1 (3.6)
Contralateral events	64 (44.8)	26 (63.4)	34 (45.9)	4 (14.3)
In situ	23 (16.0)	10 (24.3)	12 (16.2)	1 (3.6)
Invasive	29 (20.3)	9 (22.0)	19 (25.7)	1 (3.6)
Unknown	12 (8.4)	7 (17.1)	3 (4.1)	2 (7.1)
Unknown laterality	9 (6.3)	3 (7.3)	5 (6.8)	1 (3.6)
In situ	0	0	0	0
Invasive	1 (0.7)	1 (2.4)	0	0
Unknown	8 (5.6)	2 (4.9)	5 (6.8)	1 (3.6)

No second events of any kind were observed among women treated with a bilateral mastectomy ($n = 81$)

BCS breast-conserving surgery

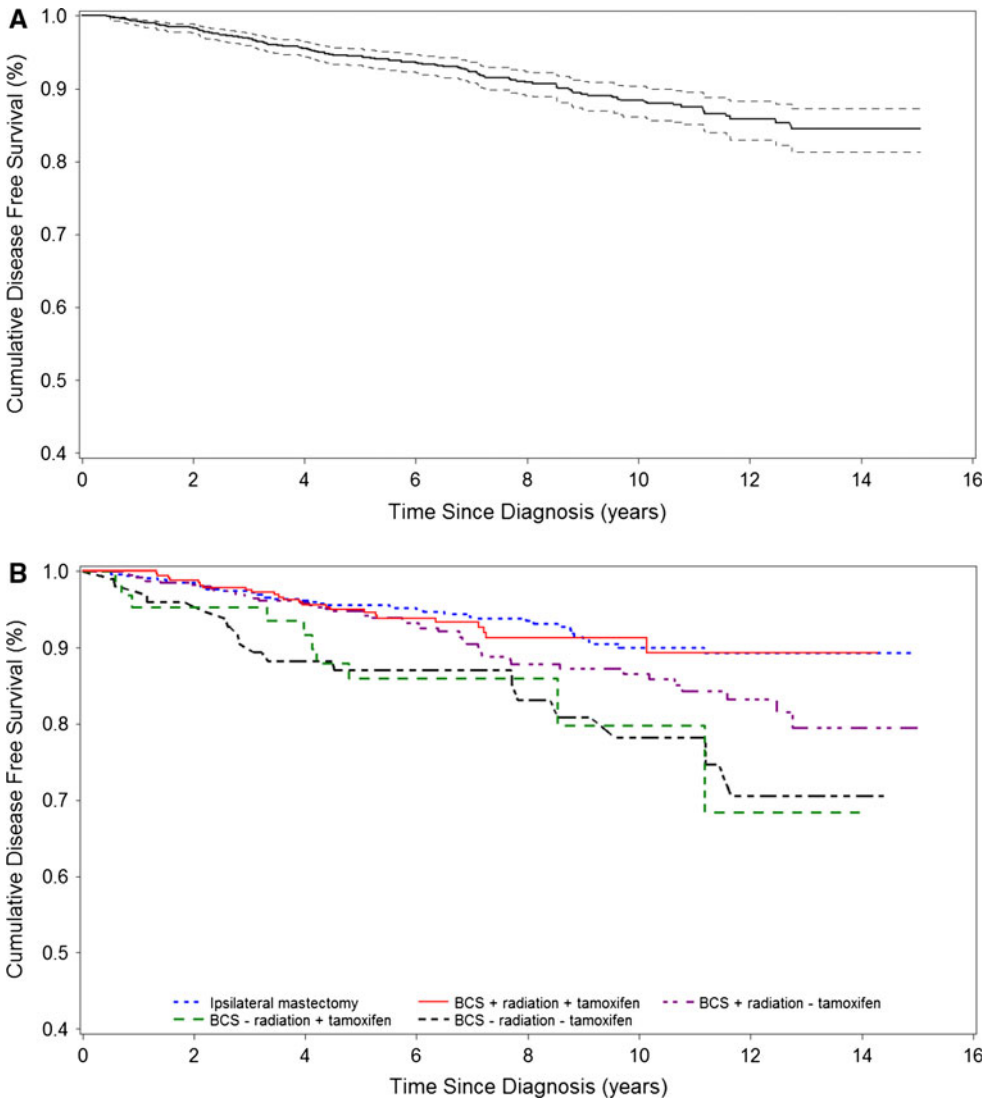

Fig. 3 **a** Overall DFS with 95 % confidence bands and **b** DFS by treatment type, Wisconsin DCIS cases ($n = 1{,}676$), 1995–2010

data not shown). The risk of a second event among women treated with BCS, radiation, and tamoxifen was comparable to that of women treated with ipsilateral mastectomy (HR 1.20, 95 % CI 0.71–2.02; Table 4).

Overall, models that adjusted only for age and year of diagnosis were similar to fully adjusted models; although hazard ratios were slightly attenuated, the estimates did not change meaningfully and relevant associations remained significant before and after complete adjustment (data not shown). Repeating the multivariable analysis using only those women with complete tumor size and grade information (approximately 30 % of the study population) and again without adjustment for tumor size or grade did not meaningfully affect the results. Point estimates for the hazard ratios remained stable and confidence intervals widened as would be expected. Likewise, the addition of BMI to the multivariable model did not have a substantive impact on the findings.

Discussion

In this large prospective population-based cohort study, approximately 95 % of subjects had not experienced a second breast cancer diagnosis at 5 years after diagnosis. Five-year survival rates for women undergoing BCS with radiation (94.8 %) were higher than for women treated with BCS without radiation (87.0 %). Women who received tamoxifen in addition to BCS and radiation had comparable 5-year DFS (95.0 %) to women receiving ipsilateral mastectomy (95.6 %).

Much of what is known about DCIS disease progression comes from randomized trials comparing various treatment protocols [10–13, 18–23]. Our study evaluated the survival experience of women with DCIS in the general population. We found that the effectiveness of radiation therapy and tamoxifen in reducing risk of second events was comparable to the efficacy observed in randomized trials. A meta-

Table 3 Five-year DFS rates according to treatment ($n = 1{,}676$), Wisconsin DCIS cases, 1995–2010

Treatment type	Any second event			Ipsilateral events									Any contralateral event		
	No. events	5 year DFS	95 % CI	Any			Invasive			In situ			No. events	5 year DFS	95 % CI
				No. events	5 year DFS	95 % CI	No. events	5 year DFS	95 % CI	No. events	5 year DFS	95 % CI			
All treatments	143[a]	94.5	93.2–95.5	70	96.9	95.9–97.7	18	99.1	98.5–99.5	30	98.5	97.7–99.0	63[a]	97.7	96.8–98.4
Bilateral mastectomy	0	100	NA	0	100	NA	0	100	NA	0	100	NA	0	100	NA
Ipsilateral mastectomy	41	95.6	93.5–97.0	12	98.7	97.3–99.4	3	99.6	98.4–99.9	0	100	NA	26	96.9	95.0–98.1
BCS with radiation[b]	73[a]	94.8	92.8–96.1	35	96.9	95.4–98.0	6	99.3	98.3–99.7	17	98.3	97.1–99.1	33[a]	98.1	96.8–98.9
No tamoxifen	46	94.8	92.1–96.6	21	97.3	95.1–98.6	4	99.2	97.4–99.7	8	98.9	97.2–99.6	21	97.9	95.9–99.0
Plus tamoxifen	24[a]	95.0	91.9–96.9	12	96.8	94.1–98.3	1	99.7	97.9–99.9	8	97.8	95.4–98.9	11[a]	98.4	96.2–99.3
BCS without radiation[c]	28	87.0	80.6–91.5	23	89.1	83.1–93.1	9	96.0	91.3–98.2	13	92.8	87.3–96.0	4	97.7	92.9–99.2
No tamoxifen	18	87.0	78.3–92.4	15	88.2	79.7–93.3	6	95.4	88.2–98.3	8	92.5	84.8–96.3	2	98.6	90.8–99.8
Plus tamoxifen	10	86.0	73.8–92.8	8	89.6	78.1–95.2	3	96.6	86.7–99.1	5	92.8	81.7–97.3	2	96.0	84.8–99.0

BCS breast-conserving surgery, *DFS* disease-free survival, *CI* confidence interval, *NA* not available

[a] Date of recurrence unknown for one participant

[b] Includes 44 women (three recurrences) with unknown tamoxifen status

[c] Includes 8 women (no recurrences) with unknown tamoxifen status

Table 4 Risk of second events according to treatment ($N = 1{,}676$), Wisconsin DCIS cases, 1995–2010

Treatment type	Any second event			Any ipsilateral event			Any contralateral event		
	No. events	HR[a]	95 % CI	No. events	HR[a]	95 % CI	No. events	HR[a]	95 % CI
Ipsilateral mastectomy	41	1.00	Ref	12	1.00	Ref	26	1.00	Ref.
BCS with radiation[b]	74	1.61	1.07–2.43	35	2.90	1.43–5.85	34	1.06	0.61–1.85
No tamoxifen	46	1.70	1.10–2.63	21	2.68	1.31–5.46	21	1.16	0.63–2.12
Plus tamoxifen	25	1.20	0.71–2.02	12	2.04	0.90–4.60	12	0.86	0.41–1.82
BCS without radiation[c]	28	2.66	1.58–4.46	23	8.77	4.03–19.1	4	0.54	0.18–1.59
No tamoxifen	18	2.59	1.45–4.62	15	8.03	3.60–17.9	2	0.42	0.10–1.82
Plus tamoxifen	10	2.69	1.30–5.57	8	7.72	3.03–19.7	2	0.79	0.18–3.45

BCS breast-conserving surgery, *HR* hazard ratio, *CI* confidence interval

[a] Multivariable model adjusts for age group, menopausal status, screening history, mode of detection, tumor size, grade, tamoxifen use, raloxifene use, aromatase inhibitor use, and year of diagnosis; analyses of BCS subgroups by tamoxifen use do not adjust for tamoxifen use

[b] Includes 44 women (3 recurrences) with unknown tamoxifen status

[c] Includes 8 women (no recurrences) with unknown tamoxifen status

analysis of randomized trials estimated that the addition of radiation therapy to BCS reduces the risk of second events by 41 % [24], which is identical to the reduction in risk of second events we observed for women treated with BCS and radiation compared to women treated with BCS without radiation. These results are also comparable to previous population-based observational studies of BCS with and without radiation [25–27]. In a study of 709 women with DCIS, Habel et al. observed a 50 % reduction in risk of a second event in the ipsilateral breast or metastasis with the use of radiation therapy (HR 0.5, 95 % CI 0.3–0.7). Warren et al. observed a similar reduction in risk with the use of radiation among 1,103 DCIS survivors included in SEER registry data (HR 0.64, 95 % CI 0.44–0.92). Our findings in a larger population of 1,676 women add strength to these earlier observational studies.

Randomized trials have also demonstrated that the addition of tamoxifen to BCS reduces risk of second events by about 30 % [10, 11, 28]. We observed a similar effect among women treated with BCS plus radiation, although the result was not statistically significant. Tamoxifen did not appear to provide a benefit for women treated with BCS without radiation in our study, though this result was based on limited numbers (total of 28 second events). In a previous population-based study of tamoxifen, Warren et al. [26] found that tamoxifen use was not associated with risk of an ipsilateral recurrence among DCIS cases who were treated with BCS. Further study is needed to more precisely establish the effectiveness of tamoxifen outside of clinical trials, with particular need to evaluate adherence to treatment and the impact of duration of treatment on DFS.

The absolute 5-year DFS rates observed in our study are substantially higher than those observed in previous randomized trials and population-based studies of cases diagnosed prior to the year 2000. The meta-analysis of randomized trials of radiation therapy (covering cases diagnosed between 1985 and 1998) estimated 5-year overall DFS of 79.3 % for women treated with lumpectomy only and 88.7 % for women treated with lumpectomy plus radiation [24]. The higher overall DFS in our study likely reflects a number of differences in the study populations, their DCIS detection, and treatments. Notably, about 40 % of the women receiving BCS in our study were also taking tamoxifen. However, for women in our study receiving tamoxifen and radiation following BCS, overall 5-year DFS was 95.0 %, which is still higher than the 91.2 % 5-year overall DFS observed in the NASBP-24 trial of tamoxifen in addition to radiation therapy [22].

The elevated DFS rates among women receiving BSC in our study likely reflect the temporal trends toward increased sensitivity of mammography and the improvements in surgical margins during BCS. This phenomenon is supported by a study of DCIS cases in three health maintenance organizations within the Cancer Research Network [29]. Among cases treated with BCS, overall 5-year DFS improved from 81.5 % for cases diagnosed in 1990–1991 to 89 % in 1998–1999. The authors determined that increasing use of radiation and tamoxifen over time could only explain about one-third of the improvement in DFS. They observed that patients diagnosed in later years were less likely to have involved surgical margins, and proposed that this likely contributed to decreased recurrence rates. Our results provide evidence that DFS rates following BCS for DCIS are continuing to increase.

No randomized trials have compared BCS to mastectomy for the treatment of DCIS, yet previous observational studies have indicated that women undergoing BCS are more likely to experience a recurrence [30–33]. In a recent analysis of DCIS cases diagnosed in western New York and Detroit between 1985 and 2000, the cumulative

incidence of ipsilateral events at 5 years was 5.5 % for women treated with BCS plus radiation and 1 % for women treated with mastectomy [32]. In our study of cases diagnosed between 1995 and 2006, this gap had narrowed to 3.1 versus 1.3 %, respectively. These results suggest that in community practice, BCS with adjuvant therapies now provides very similar DFS to ipsilateral mastectomy.

Like other population-based studies, our study has limitations that must be considered. Although participation in our study by eligible women was high, it is possible that women in active treatment for a breast cancer recurrence may have differentially chosen not to participate. Our multiple rounds of follow-up interviews and high participation rates ameliorate this concern somewhat. The high DFS observed in our study resulted in low power to detect differences in stratified analyses. We relied on self-report of treatments received and second event diagnoses. Medical record validation indicated excellent reliability of self-reported second events (91 % confirmed). Thus, the inclusion of second event diagnoses which could not be confirmed via medical records is not likely to have introduced substantial bias to our results. Sensitivity analyses indicated that the associations between each treatment and DFS were not meaningfully changed when the unconfirmed second events were excluded from the analyses.

The effectiveness of BCS for DCIS varies according to margin status [32] and it is known that the threshold for adequate margins varies widely in community practice, resulting in highly variable re-excision rates [34]. We were unable to examine the role of margin status or other surgical practice factors in our results. We were also unable to control for estrogen and progesterone receptor status, as this data was unavailable for the index DCIS diagnoses. In addition, we had incomplete data on covariates such as tumor size and grade. To enable statistically efficient use of the data and avoid bias by excluding cases with missing information, we used multiple imputation to estimate missing data for these covariates [35].

Our results are based on outcomes over an average of 7.1 years (range 0.4–15.1 years) of follow-up. It is possible that different patterns in DFS by treatment may emerge with longer follow-up. However, prior studies of DCIS outcomes according to treatment have generally reported similar proportional differences at 5 and 10 years of follow-up [10, 32]. WISC study subjects will continue to be followed to compare long-term outcomes among women treated with mastectomy and BCS with adjuvant therapies. Finally, we note that the study population was predominantly white (95 %), reflecting the racial distribution of breast cancer cases diagnosed in Wisconsin. Studies in more racially diverse populations will be needed to assess the generalizability of these results.

There are approximately 500,000 women alive today in the United States with a diagnosis of DCIS [36]. These women face an increased risk of developing an invasive breast cancer diagnosis [37] and may suffer reductions in their health-related quality of life due to side effects of treatments and the psychological effects of a cancer diagnosis [38]. Our findings provide evidence regarding the comparative effectiveness of current treatments for DCIS in community practice. The results suggest that BCS with radiation and tamoxifen can provide DFS rates comparable to that of ipsilateral mastectomy.

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481-S

TUMOR MARKERS IN RELATION TO DISEASE-FREE SURVIVAL AMONG WOMEN WITH DUCTAL CARCINOMA IN SITU OF THE BREAST. *Alex Binder, John Hampton, Brian Sprague, Matthew Walsh, Andreas Friedl, Polly Newcomb, Amy Trentham-Dietz (University of Wisconsin-Madison, Madison WI 53726)

Ductal carcinoma in situ (DCIS) constitutes approximately 20% of all new breast cancer diagnoses. As there is no definitive means to determine which cases of DCIS will lead to invasive breast cancer, prognostic markers are needed. To examine the relation between disease-free survival and molecular markers, we prospectively identified new cases of DCIS diagnosed between 1997 and 2000 in Wisconsin women 18-74 years of age from the state cancer registry. Cases completed baseline risk factor interviews about 1 year after initial diagnosis, and provided consent to access medical records and tumor blocks. Tumor markers including Estrogen Receptor (ER), Progesterone Receptor (PR), HER2/neu, Ki-67, and p53 were evaluated using immunohistochemistry. Follow-up interviews gathered information on subsequent breast cancer diagnoses including cancer treatment. Among the 245 DCIS cases, 36 (15%) had a second breast cancer diagnosis (86% confirmed by pathology reports). Median follow-up time through the date of a second breast cancer diagnosis or last interview was 11.2 years (range 0.5-15.0 years). Hazard rate ratios (HR), 95% confidence intervals (CI), and p-values were calculated to compare the occurrence of a second breast cancer diagnosis according to tumor markers using Cox proportional hazards models adjusted for age and treatment. An association was observed between lack of p53 overexpression and a second diagnosis (14% versus 42% for p53-positive versus negative; HR 2.63, 95% CI 1.13, 6.12; $P = 0.02$). Hazard ratios for the other markers were not significant (ER, $P = 0.52$; PR, $P = 0.09$; HER2, $P = 0.64$; Ki-67, $P = 0.20$). These results suggest that the absence of p53 protein in DCIS tumors is a risk factor for a second breast cancer diagnosis. Additional analyses will consider risk factors together with tumor and treatment factors in relation to outcomes after a DCIS diagnosis.

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NOVEL METHODOLOGIES TO ADDRESS MOLECULAR HETEROGENEITY OF DISEASE PROCESSES IN EPIDEMIOLOGIC RESEARCH. *Aya Kuchiba, Molin Wang, Shuji Ogino, Donna Spiegelman (Harvard School of Public Health, Boston MA 02115)

Epidemiologic research typically investigates the associations between exposures and the risk of a disease, in which the disease of interest is treated as a single outcome. However, many human diseases, including colon cancer, type II diabetes mellitus and myocardial infarction, are comprised of a range of heterogeneous molecular and pathologic processes, likely reflecting the influences of diverse exposures. The approach, which incorporates data on the molecular and pathologic features of a disease directly into epidemiologic studies, Molecular Pathological Epidemiology, has been proposed to better identify causal factors and better understand how potential etiologic factors influence disease development. In this study, we present statistical methods for evaluating whether the effect of a potential risk factor varies by subtypes of the disease, in cohort studies, case-control studies and case-case study designs. A new SAS macro is presented, %subtype, to implement these methods. This macro tests overall heterogeneity through the common effect test (i.e., the null hypothesis is that all of the effects of exposure on the different subtypes are the same) as well as pair-wise differences in exposure effects. In adjusting for confounding, the effects are allowed to vary for the different subtypes or they can be assumed to be the same across the different subtypes. To illustrate our methods, we apply %subtype to the study of the effect of alcohol intake on LINE-1 methylation subtypes of colon cancer in the Health Professionals Follow-up Study, where 51,529 men have been followed since 1986 during which time 268 cases of colon cancer have occurred. Results are presented for all 3 possible study designs for comparison purposes.

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BREAST CANCER SUBTYPES AND PREVIOUSLY ESTABLISHED GENETIC RISK FACTORS: A BAYESIAN APPROACH. *Katie O'Brien, Stephen Cole, Lawrence Engel, Jeannette Bensen, Charles Poole, Amy Herring, Robert Millikan (UNC-Chapel Hill, Chapel Hill NC 27599)

Gene expression analyses indicate that breast cancer is a heterogeneous disease with at least 5 immunohistologic subtypes. Despite growing evidence that these subtypes are etiologically and prognostically distinct, few studies have investigated whether they have divergent genetic risk factors. To help fill in this gap in our understanding, we examined associations between breast cancer subtypes and previously established susceptibility loci among white and African-American women in the Carolina Breast Cancer Study. We used Bayesian polytomous logistic regression to estimate odds ratios (ORs) and 95% posterior intervals (PIs) for the association between each of 78 single nucleotide polymorphisms (SNPs) and 5 breast cancer subtypes. Subtypes were defined using 5 immunohistochemical markers: estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptors 1 and 2 (HER1/2) and cytokeratin (CK) 5/6. Several SNPs in TNRC9/TOX3 were associated with luminal A (ER/PR+, HER2-) or basal-like breast cancer (ER-, PR-, HER2-, HER1 or CK 5/6+), and one SNP (rs3104746) was associated with both. SNPs in FGFR2 were associated with luminal A, luminal B (ER/PR+, HER2+), or HER2+/ER-disease, but none were associated with basal-like disease. We also observed subtype differences in the effects of SNPs in 2q35, 4p, TLR1, MAP3K1, ESR1, CDKN2A/B, ANKRD16, and ZM1Z1. We found evidence that genetic risk factors for breast cancer vary by subtype and further clarified the role of several key susceptibility genes.

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CHANGE OF MAMMOGRAPHIC DENSITY PREDICTS THE RISK OF CONTRALATERAL BREAST CANCER. *Maria EC Sandberg, Jingmei Li, Per Hall, Mikael Hartman, Isabel dos-Santos-Silva, Keith Humphreys, Kamila Czene (Karolinska Institutet, Stockholm Sweden)

Introduction: Mammographic density is a strong risk factor for breast cancer, but it is unknown whether density at first breast cancer diagnosis and changes during follow-up influences risk of non- simultaneous contralateral breast cancer (CBC). Methods: We collected mammograms for CBC-patients (cases) and unilateral breast cancer patients (controls), individually matched on age and calendar period of first breast cancer diagnosis, type of adjuvant therapy and length of follow-up. The odds of CBC as a function of changes of density during follow-up were investigated using conditional logistic regression, adjusting for non-dense area at diagnosis. Results: Patients who experienced $\geq$10% absolute decrease in percent density had a 55% decreased odds of CBC (Odds Ratio (OR) = 0.45 95% CI: 0.24-0.84) relative to patients who had little or no change in density from baseline to first follow-up mammogram (mean = 1.6 (standard deviation = 0.6) years after diagnosis), whereas among those who experienced an absolute increase in percent density there was little change in the odds of CBC (OR = 0.83 95% CI: 0.24-2.87). Conclusion: Decrease of mammographic density within the first two years after a first diagnosis is associated with a significantly reduced risk of CBC. This potential new risk predictor can thus contribute to decision making as well as provide reassurance to the patients at decreased risk.

ation and other classical reproductive and hormonal breast cancer risk factors were observed.

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Lifestyle Factors and the Risk of a Second Breast Diagnosis after DCIS in the Wisconsin In Situ Cohort

McLaughlin V, Trentham-Dietz A, Hampton JM, Newcomb PA, Sprague BL

Purpose: Certain tumor factors have been associated with increased likelihood of a second breast diagnosis after treatment for ductal carcinoma in situ (DCIS) breast cancer. However, little information exists on modifiable lifestyle factors that affect prognosis after DCIS and may be useful for survivors in reducing their risk of a second breast cancer event. Methods: We examined the longitudinal association between body mass index (BMI), physical activity, and alcohol intake and risk of a second breast diagnosis among 1,925 DCIS survivors first diagnosed in 1997–2006 and enrolled in the Wisconsin In Situ Cohort. Data were collected during biennial patient interviews and diagnosis information was validated via pathology report. BMI, physical activity, and alcohol intake were examined over time using Chi-square and ANOVA methods. Cox proportional hazards regression was used to estimate the risk of a second diagnosis after adjustment for patient, tumor, and treatment factors. Repeated measures were incorporated to make use of exposure measurements taken at each post-diagnosis interview. Results: Over an average of 6.6 years of follow-up, 162 second breast cancer diagnoses were reported. Significant trends of increasing BMI and decreasing physical activity were observed over time since diagnosis ($p < 0.001$). For all women, a significant linear trend of increasing risk of a second diagnosis was found over increasing categories of post-diagnosis alcohol intake (p-trend 0.02). Among women treated with ipsilateral mastectomy, a reduction in risk was suggested with increasing post-diagnosis physical activity (HR 0.67, 95% CI 0.45, 1.02 for each additional hour/week). Among postmenopausal women, higher categories of post-diagnosis BMI were associated with increasing risk, although these results were of borderline significance (p-trend 0.09). Conclusion: This study is the first to examine the association of physical activity and alcohol intake with second breast diagnoses in an exclusively DCIS population. Our results suggest that DCIS survivors may reduce their risk of a second diagnosis by engaging in physical activity and reducing their alcohol consumption.

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A Prospective Study of Circulating Adipokine Levels and Risk of Multiple Myeloma

Hofmann J, Liao L, Pollak M, Wang Y, Pfeiffer R, Baris D, Andreotti G, Lan Q, Landgren O, Rothman N, Purdue M

Purpose: Obesity is associated with an increased risk of multiple myeloma (MM), although the biologic mechanisms underlying this association are unclear. We conducted a nested case-control study in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial to evaluate the hypothesis that altered circulating levels of adipokines, polypeptide hormones with pro- and anti-inflammatory properties secreted by adipose tissue, may partly explain the association between obesity and MM. Methods: We investigated whether circulating levels of leptin, total adiponectin, and high-molecular-weight (HMW) adiponectin are associated with MM among 174 cases and 348 controls in PLCO. Two controls were matched to each case on age at baseline, sex, race, date of phlebotomy, time of day of phlebotomy, and study year of specimen collection. Plasma adipokine concentrations were measured by enzyme-linked immunosorbent assay; overall coefficients of variation were $\leq$8.5%. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using conditional logistic regression. Results: Inverse associations with MM were observed for total adiponectin (highest quartile vs. lowest: OR = 0.49, 95% CI = 0.26–0.93, P-trend = 0.03) and HMW adiponectin (OR = 0.44, 95% CI = 0.23–0.85, P-trend = 0.01). These associations remained after adjusting for body mass index (BMI), stratifying by sex, and restricting to cases diagnosed approximately eight years or more after blood collection. We observed a modest association between BMI and MM (OR per 5 kg/m^2 increase = 1.14, 95% CI = 0.94–1.39), which was attenuated by approximately 40% after adjusting for adiponectin. Leptin levels were not associated with MM. Conclusions: These results suggest that higher circulating levels of adiponectin are protective against MM, and that adiponectin may play an important role in obesity-related myelomagenesis. This study is, to our knowledge, the first prospective investigation of circulating adipokines and MM. Our findings are particularly intriguing in light of recent evidence that host-derived adiponectin is tumor-suppressive and a potential novel therapeutic target for MM and associated bone disease.

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Aspirin and Colorectal Cancer Incidence and Mortality by CTNNB1 Expression: A Molecular Pathological Epidemiology (MPE) Study

Sun R, Nishihara R, Qian ZR, Chan AT, and Ogino S

Purpose: Experimental studies showed that aspirin down-regulates the WNT/CTNNB1 (β-catenin) signaling

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Title: Alteration of stromal collagen fiber orientation in DCIS

Body: Approximately 20% of new diagnoses of breast cancer are ductal carcinoma in situ (DCIS), a non-invasive form of breast cancer. Treatment decision-making for DCIS is challenging since current predictors of disease-free survival are limited, so that most women are presented with options for surgery, radiation and tamoxifen – all options with consequences for quality of life. Prior studies of prognostic factors for DCIS have focused on morphologic, genetic, and protein expression patterns of the DCIS cells. However, laboratory evidence suggests that the tumor microenvironment may play a key role in tumor invasion and progression. Collagen is the most abundant component of the stroma surrounding the breast ducts in which cancers develop. We previously observed that, in invasive breast cancer, tumors with greater numbers of collagen fibers aligned perpendicularly from the tumor were more likely to predict poor survival than tumors with collagen fibers in primarily parallel patterns near the tumor boundary (Conklin Am J Pathol 2011). To improve our ability to predict breast cancer outcomes in women with DCIS, we examined the alignment of collagen adjacent to ducts affected by DCIS to test whether alignment patterns were similar to patterns observed in tissue labeled as “normal” from biopsy and surgical sections. We evaluated collagen alignment in 255 Wisconsin women diagnosed with DCIS in 1997-2000 and followed for a median of 11.2 years (range 1-15). Stromal collagen alignment was evaluated from routine H&E tissue slides prepared at the time of diagnosis using second harmonic generation (SHG) microscopy, a label-free multiphoton laser scanning technique that selectively images collagen. SHG images were acquired and evaluated for 3-5 regions on each DCIS and normal slide for each patient; the angles of collagen fibers with respect to the DCIS lesion/stroma boundary were calculated using customized imaging software. Data for the distribution of angles were compared for normal ducts and DCIS lesions using compositional data analysis with the number of fibers totaled according to 5-angle bins (1-5, 6-10, 11-15, …, 86-90 degrees). Repeated measures linear

regression models were fit to log-transformed ratios of binned counts as a function of tissue type. Dependence among repeated counts within a single region was modeled using an unstructured variance-covariance matrix. Dependence among measurements within a single subject was modeled using a compound symmetry correlation structure. Overall, the distribution of collagen fiber angles from DCIS lesions differed significantly (P=0.0002) from the distribution of collagen fibers surrounding normal ducts. Collagen fibers surrounding DCIS lesions were 11-18% more likely to orient at 75-90 degrees relative to the lesion boundary than fibers surrounding normal ducts; fibers were more similarly aligned in both DCIS lesions and normal ducts at other smaller angles. These results underscore the relevance of the tumor microenvironment, in particular the arrangement of the collagen fiber matrix. Planned data analysis will next examine whether collagen fiber alignment patterns differ between DCIS patients who did and did not experience a second breast cancer diagnosis over the course of follow-up.

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